Do grassland plant communities profit from N partitioning by soil depth?

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Abstract: Recent biodiversity–ecosystem functioning experiments in temperate grasslands have shown that productivity positively correlates with plant species richness. Resource partitioning (in particular, nitrogen [N] partitioning) has been proposed as one possible mechanism to explain this pattern. There is evidence for interspecific differences in chemical form, soil depth, and timing of N uptake. However, it has rarely been tested whether such differences result in increased N exploitation at the plant community level. Using 15N-labeled litter that was mixed into different soil layers, we tested whether eight common grasses and forbs grown in communities of one, two, or four species differ with respect to the proportions of N taken up from different soil depths (N niche), and how this affects the total N uptake of plant communities. We calculated proportional similarity between species (niche overlap) with regard to N uptake from the labeled soil layers; we further calculated an a priori measure of community N uptake based on species N uptake in monoculture (community niche). Interestingly, however, plant community N uptake was not affected by species richness, possibly because community-level N uptake was determined by (diversity-independent) soil N mineralization rates. We nevertheless observed a positive effect of species richness on productivity due to increased aboveground biomass : N ratios. This may indicate increased competition for light, resulting in increased amounts of comparably N-poor stem tissue. However, community N content and biomass were positively correlated with the community niche, a measure which is strongly linked to species composition. Thus, our results suggest that the studied species are generalists rather than specialists regarding N uptake depth, and that species composition was more important than species richness in determining community N uptake. Overall, N partitioning may be a less important driver of positive biodiversity–productivity effects in temperate grasslands than previously assumed.

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Do grassland plant communities profit from N partitioning by soil depth?

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Abstract

Recent biodiversity–ecosystem functioning experiments in temperate grasslands have shown that productivity positively correlates with plant species richness. Resource partitioning—in particular nitrogen (N) partitioning—has been proposed as one possible mechanism to explain this pattern. There is evidence for interspecific differences in chemical form, soil depth and timing of N uptake. However, it has rarely been tested whether such differences result in increased N exploitation at the plant community-level. Using ^{15}N-labeled litter which was mixed into different soil layers, we tested whether eight common grasses and forbs grown in communities of one, two or four species differ with respect to the proportions of N taken up from different soil depths (N-niche), and how this affects the total N uptake of plant communities. We calculated proportional similarity between species (niche overlap) with regard to N uptake from the labeled soil layers; we further calculated an *a priori* measure of community N uptake based on species N uptake in monoculture (community niche). Interestingly, however, plant community N uptake was not affected by species richness, possibly because community-level N uptake was determined by (diversity-independent) soil N mineralization rates. We nevertheless observed a positive effect of species richness on productivity due to increased aboveground biomass:N ratios. This may indicate increased competition for light resulting in increased amounts of comparably N-poor stem tissue. However, community N content and biomass were positively correlated with the community niche, a measure which is strongly linked to species composition. Thus, our results suggest that the studied species are generalists rather than specialists regarding N uptake depth, and that species composition was more important than species richness in determining community N uptake. Overall, N partitioning may be a less important driver of positive biodiversity–productivity effects in temperate grasslands than previously assumed.

Keywords ^{15}N, biodiversity–ecosystem functioning, niche, nitrogen, proportional similarity, resource partitioning, rooting depth, species richness, temperate grassland

Introduction

The past two decades have seen a burst of studies addressing the relationship between biodiversity and ecosystem functioning. Experiments in temperate grasslands have repeatedly shown a positive effect of plant species richness on productivity (reviewed e.g., in Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2007, Duffy 2009, Naeem et al. 2009, Cardinale et al. 2011). Resource partitioning has often been proposed as a mechanism explaining this relationship. Interspecific differences in resource niches should lead to more complete use of available resources in more diverse plant communities. This has revived an old idea in ecology, going back to Darwin: niches may not only explain species coexistence but also affect ecosystem functioning, through the ecological "division of labour" (Darwin 1985, Hector and Hooper 2002).

Nitrogen (N) availability limits primary productivity in many ecosystems including temperate grasslands (Vitousek and Howarth 1991), suggesting that N uptake from soil may be an ideal test case for the presence of interspecific resource partitioning by plants. Several studies have investigated partitioning of N with respect to chemical form (such as NH_4^+, NO_3^-, and organic N) using ^{15}N-labeling techniques (McKane et al. 2002, Weigelt et al. 2005, Miller et al. 2007, Pornon et al. 2007). Plant species differ in rooting depth (Parrish and Bazzaz 1976, Berendse 1982), depth of root activity (Veresoglou and Fitter 1984, Fitter 1986, Mamolos et al. 1995) and depth of water uptake (Gordon and Rice 1992, Nippert and Knapp 2007). However, only a few studies have investigated partitioning of N by soil depth: McKane et al. (2002) observed that arctic tundra plants simultaneously partitioned N by depth, chemical form and time, whereas Kahmen et al. (2006) found no evidence for partitioning of soil N by depth. Moreover, while there is good evidence that plants vary in

their capacity to assimilate different chemical forms of N or to exploit different soil depths, it has hardly been tested whether such differences change with species richness and whether they lead to more complete N exploitation in species rich communities.

If a mixture is composed of "specialist" species that differ inherently in resource-use patterns, then the mixture should exploit resources more completely than any of the monocultures (in an extreme case the resource capture of complete specialists would be additive). In contrast, if a mixture is composed of "generalist" species that largely overlap in resource use, the mixture would not be expected to exploit more resources than the monocultures. This has been demonstrated elegantly for animals, with specialist and generalist parasitoids (Finke and Snyder 2008), and for bacterial strains (Gravel et al. 2011). Alternatively, species may be able to use a broad range of resources but may behave opportunistically rather than occupying a defined niche. They may behave as generalists under intraspecific competition (in monocultures) but as specialists in the presence of interspecific competition (in species mixtures). Berendse (1982) showed that *P. lanceolata* acquired nutrients from deeper soil layers when grown with the shallow-rooting grass *Anthoxanthum odoratum* than when grown in monocultures. However, a change from generalist to specialist behaviour does not necessarily imply that community-level resource uptake increases with diversity. A more rigorous investigation of the relation between resource partitioning and resource use requires investigating the species' realised resource niches while manipulating species richness.

Neighbor removal experiments have investigated partitioning between chemical forms of N in the presence and absence of interspecific competition (Miller and Bowman 2002, Ashton et al. 2008). In an earlier experiment in the field, we assessed plant uptake of three chemical forms of N from two soil depths using ^{15}N-tracer solutions (von Felten et al. 2009). We found a decrease in niche overlap among species across three levels of species richness. However, these studies could not relate interspecific niche differentiation between species to community N use, either because plant species richness was confounded with plant density (Miller and

Bowman 2002, Ashton et al. 2008), or because community-level N acquisition was not measured (von Felten et al. 2009).

We conducted a ^{15}N-labeling experiment to test for interspecific differences in N uptake from deep and shallow soil among temperate grass and non-legume forb species. Unlike other studies on N partitioning, using liquid tracers, we mixed ^{15}N-labeled litter into the soil, allowing a more precise and homogeneous distribution and microbial mobilisation of the label. We further tested whether N uptake niches (quantified as the fraction of N acquired from separate soil layers) and their overlap (quantified as proportional similarity, Colwell and Futuyma 1971, Feinsinger et al. 1981) depended on species richness. Finally, we tested whether plant community N uptake in mixtures increased with species richness and whether it can be predicted from the "community niche" (Salles et al. 2009), an *a priori* index based on N uptake of the component species in monoculture.

Methods

Experimental Design

We set up a factorial mesocosm experiment combining a plant species richness and a soil labeling treatment. The species richness treatment consisted of growing all possible communities of one, two and four species that could be assembled from two separate pools of four species (22 communities; Table 1). Each pool contained two common grasses and forbs. Working with two non-overlapping species pools allows more generalizable results that are not restricted to a particular species pool. The soil labeling treatment consisted of mixing ^{15}N-labeled litter with the top soil layer (0-20 cm, "shallow" soil layer treatment) or the bottom soil layer (20-40 cm, "deep" soil layer treatment). Additional mesocosms without soil ^{15}N labeling were established outside the experiment to determine natural background ^{15}N abundances in plant biomass.

The experimental communities were set up in boxes of 40 cm × 60 cm area and 40 cm depth. These boxes were subdivided into eight (2 × 4) rectangular compartments. The experimental treatments were applied in a "split-plot" design: one plant community of a given composition was grown in each box (all eight compartments) and soil labeling treatments were applied to half a box (four adjacent compartments). The four compartments of half a box were used for destructive harvests.

Set-up of Mesocosms

Our experiment was set up in the experimental garden of the Institute of Environmental Sciences, at the University of Zurich (Switzerland). Mesocosms were built from polypropylene boxes that were subdivided using waterproof polyphenol-resin-coated boards. Individual compartments (18 cm × 13.5 cm) were sealed using silicone and polyurethane lute to avoid any transfer of ^{15}N to neighboring compartments. Each compartment had four 10 mm drainage holes at the bottom and was fitted with a drainage mat (Enkadrain, Colbond, Arnhem, The Netherlands). The boxes were insulated using 2 cm thick styrofoam boards to minimize warming by sunlight.

Each compartment was filled with sieved, natural field soil (0.35±0.01 % N and 3.6±0.04 % C, pH = 7.6) that was compressed to prevent subsequent settling of the soil. 1.4 g ^{15}N-labeled *Festuca rubra* material containing 2.5 mg ^{15}N (1.4 g *F. rubra* × 1.18 % N × 15 atom% ^{15}N) was mixed either into the deep (lower 20 cm), the shallow (upper 20 cm), or no soil layer. The same amount of non-labeled *F. rubra* material was mixed into all non-labeled layers. The *F. rubra* material was obtained by growing plants for 11 weeks on quartz sand supplied with nutrient solution. The nutrient solution contained ^{15}N-labeled KNO_3 (15 atom% ^{15}N) or KNO_3 at natural abundance levels to produce ^{15}N-labeled and non-labeled litter. The plants were cut to the ground, dried and shredded into pieces of ca. 2 cm.

From June 7 to 10, 2005, twelve five-week old seedlings were transplanted into each box compartment. The seedlings were organised in three rows with four individuals each; the

middle row was offset by half the distance between individuals, i.e. plantlets arranged on a hexagonal grid with constant spacing between individuals. Each row contained the full species set present in the respective community, with species positions randomized within rows. Plant communities were weeded regularly and were watered daily with an automated irrigation system except for rainy days. Daily average temperatures at the site (502 m asl) ranged from -9.1°C (January 29, 2005) to 26.1 °C (July 28, 2005).

Harvests and sample preparation

Box compartments were destructively harvested after nine weeks (8–21 August 2005), after 15 weeks (19 September–7 October 2005) and after 11 months (15–29 May 2006). In addition, plant biomass in all remaining compartments was cut to 2 cm at 15 weeks (end of the growing season 2005). For each destructive harvest, two compartments per box were randomly selected (one from each ^{15}N-treatment). At the same time, one compartment to which no ^{15}N-label had been added was harvested to assess background ^{15}N abundances to calculate ^{15}N enrichment.

Aboveground biomass was harvested at the species level. Roots were washed on a 2 mm sieve. We attempted to also retrieve roots at the species level by extracting individuals separately, but the proportion of residual roots that could not be allocated to species was very large. We thus only present root data at the community level. All plant material was dried (80°C, 48 h) and weighed. All samples were ground and N and ^{15}N measured by isotope ratio mass spectrometery (Delta^{plus} XP IRMS, Finnigan MAT, coupled to a Flash EA 1112 NC elemental analyzer, CE Instruments).

The consistency and spatial distribution of the ^{15}N-labeling treatments were assessed by measuring δ^{15}N in extractable soil N (n=72 measurements, 6 compartments × 2 treatments × 2 depths at each of three harvests). For each sample, 15 g fresh soil sieved to 2 mm were extracted with 50 ml 0.03 M K_2SO_4 for 30 min. ^{15}N was measured in freeze-dried extracts by isotope ratio mass spectrometry (Delta^{plus} XP IRMS, Finnigan MAT, coupled to a EuroEA

174 3000 elemental analyzer, HEKAtech).

175 **Data analysis**

176 For each sample we calculated ^{15}N tracer concentration ([$^{15}N_{ex}$]; atom% excess or mg excess
177 ^{15}N per g N) and ^{15}N tracer content ($^{15}N_{ex}$; g excess ^{15}N).

178 Then, for each population, the fraction of ^{15}N tracer taken up from either deep soil (deep
179 fraction, DF) or shallow soil (shallow fraction, SF) was calculated,

$$DF = \frac{[^{15}N_{ex}]_{deep}}{[^{15}N_{ex}]_{deep} + [^{15}N_{ex}]_{shallow}} \quad (1)$$

$$SF = 1 - DF \quad (2)$$

180 where [$^{15}N_{ex}$]$_{deep}$ and [$^{15}N_{ex}$]$_{shallow}$ are aboveground ^{15}N tracer concentrations from a pair of
181 compartments with the respective ^{15}N treatments (n=2 pairs per population and harvest).

182 To quantify niche overlap, we calculated the proportional similarity index (Colwell and
183 Futuyma 1971, Feinsinger et al. 1981) based on DF and SF, i.e. n=2 N sources, between
184 pairs of species (indicated as species 1 and 2):

$$PS = 1 - 0.5 \sum_{i=1}^{n} |p_{1i} - p_{2i}| \quad \text{(general form)} \quad (3)$$

$$PS = 1 - 0.5\,(|DF_1 - DF_2| + |SF_1 - SF_2|) \qquad (n{=}2) \quad (4)$$

185 Values of proportional similarity (PS) range from zero to complete overlap (=1) between
186 species. For each species pool, proportional similarity was calculated between all species
187 when grown in monoculture (six pairwise combinations). Within mixtures, proportional
188 similarity was calculated between pairs of species (one combination in 2-species mixtures, six
189 combinations in 4-species mixtures).

190 To quantify the niche space occupied by the whole plant community, we calculated the
191 expected "community niche" (CN, Salles et al. 2009). CN *a priori* predicts the N uptake of
192 a mixture by summing the maximum observed monoculture N uptake from deep and shallow

soil across species present in the mixture:

$$CN = \sum_{i=1}^{n} max\left(P_{i,1}, ..., P_{i,m}\right) \quad \text{(general form)} \quad (5)$$

$$CN_{depth} = max_{deep}({}^{15}N_{ex,1}, ..., {}^{15}N_{ex,m}) + max_{shallow}({}^{15}N_{ex,1}, ..., {}^{15}N_{ex,m}) \qquad (n{=}2) \qquad (6)$$

where n is the number of resources (here: n=2 soil depths), m is the number of species in the mixture (here: m=1,2, or 4) and P_{ij} is the performance of species j on soil depth i. Here, ${}^{15}N_{ex,j,deep}$ and ${}^{15}N_{ex,j,shallow}$ is the total (above- and belowground) ^{15}N uptake of species j in the deep and the shallow ^{15}N treatment in monoculture (mean of two replicates per monoculture × ^{15}N treatment × harvest combination). CN of each plant community combines the maximum N uptake from shallow and deep soil in monoculture across all species present in the community. We also included values of CN for monocultures in our analyses (although these were not calculated *a priori*), to account for effects of CN at all levels of species richness. This yielded 66 values of CN, 22 species compositions (Table 1) × three harvests.

Data were analyzed using linear mixed effects model ANOVA since we used a hierarchical experimental design (function lme in R version 2.11.0, R Development Core Team 2010). For the analysis of ^{15}N enrichment of aboveground biomass (n=576, 192 populations × 3 harvests) we fitted harvest, ^{15}N treatment and the interaction term as fixed factors. Treatment unit and compartment were used as random factors. The deep fraction of tracer uptake (DF) was analyzed seperately for monocultures and mixtures. For the analysis of DF in monocultures (n=48, 8 species × 3 harvests × 2 replicates) we fitted species, harvest and the species × harvest interaction as fixed factors. Species pool and the compartment pair (see calculation of DF) were used as random factors. For the analysis in mixtures (n=240, [12 compositions × 2 species × 2 replicates + 2 compositions × 4 species × 4 replicates] × 3 harvests), we additionnally fitted species richness (two vs. four species) and included all interaction terms (see Table 2). In addition, we fitted a second model with the species term

replaced by functional group (grasses vs. forbs). Species pool, species composition and the compartment pair were used as random factors. Because the errors did not significantly deviate from normality, there was no need to transform DF. In addition, we calculated the Spearman rank correlation coefficient between species ranks in DF and aboveground biomass within mixtures (ranks 1–4 in 4-species mixtures and ranks 1–2 in 2-species mixtures).

Proportional similarity was arc sine square root transformed to meet the assumption of normal errors. We fitted the fixed factors species richness (three-level factor and log-linear effect), species pair, harvest and the interactions species richness × harvest and species pair × harvest (Table 3). Random factors were species pool, species composition and the specific species pair.

To assess the effect of species richness on aboveground biomass and N content on the community level, we used a model with pool and species composition as random factors, and species richness (log-linear effect), harvest and the interaction term as fixed factors. Similarly, we tested the effect of the "community niche" (CN) on aboveground biomass and aboveground N content. CN was fitted as continuous explanatory variable instead of species richness. We used pool and composition × harvest as random factors, since CN is defined per composition and harvest. Note that mean numbers given in the text are always mean±SE.

Results

^{15}N labeling of soil

The ^{15}N-labeling treatments were stable across harvests; average soil δ^{15}N was 45.0±1.8 ‰ in the upper layer and 10.7±0.3 ‰ in the lower layer for the shallow ^{15}N treatment, and 8.5±0.2 ‰ and 46.4±1.5 ‰ for the deep ^{15}N treatment, respectively. These δ^{15}N values indicate that unlabeled layers remained unlabeled (background δ^{15}N of soil: 10.9±0.3 ‰) for the duration of the experiment (11 months), and that the ^{15}N enrichment was similar for

both treatments.

Addition of ^{15}N labeled plant material led to considerable enrichment of plant aboveground ^{15}N, which was higher in compartments with the shallow than with the deep ^{15}N treatment across all populations (Appendix Tables 1 and 2). As a consequence, the fraction of ^{15}N tracer taken up from deep soil (deep fraction, DF) was on average lower than 0.5 (mean=0.36±0.007). Note that the shallow fraction SF=1-DF (Eq. 2).

^{15}N uptake from deep and shallow soil in monocultures

When grown in monoculture, species did not differ in the fraction of ^{15}N tracer taken up from deep soil (DF, see Appendix Fig. 1 and Table 3). However, the deep fraction increased across harvests from a mean of 0.31±0.02 in August 2005 to 0.43±0.01 in May 2006 ($F_{2,16}$=17.8, P<0.001), most likely due to deeper root penetration over time. This might be due to depletion of nutrients in the shallow soil layer, as indicated by decreasing ^{15}N enrichment of aboveground biomass on the shallow treatment but stable ^{15}N enrichment on the deep treatment (harvest × ^{15}N treatment interaction, Appendix Table 2).

Consistent with no difference in DF among species, values of proportional similarity (PS) were large, with an overall mean of 0.94±0.01. PS was ≥0.8 between all but one species pair at harvest 1 (0.74 between *L. perenne* and *T. officinale*, but PS of the second replicate was 0.93), and always ≥0.85 at harvests 2 and 3 (Fig. 1).

^{15}N uptake from deep and shallow soil in mixtures

When grown in mixtures of two or four species, species differed significantly in the fraction of ^{15}N tracer taken up from deep soil, but species differences changed over time (species × harvest interaction, Table 2 and Appendix Fig. 1). Grasses took up more ^{15}N from deep soil than forbs at harvests 1 and 2, whereas the forbs took up more ^{15}N from deep soil than the grasses at harvest 3 (functional group × harvest interaction, Appendix Table 4). It seemed that changes in functional group effects over time were more pronounced than changes in species effects. However, a model comparison based on AIC and a likelihood ratio test

indicated that the model including species effects fit the data better than the model including functional groups instead of species (Likelihood-ratio=116.9, $P<.001$). As for the monocultures, DF generally increased over time (across harvests) in mixtures. However, the species richness of mixtures did not affect DF.

We found a positive correlation between the aboveground biomass of plant populations and the fraction of N taken up from deep soil (DF), which means that plant species with high biomass in mixture took up more N from deep soil than species with low biomass in mixture (Spearman rank correlation coefficient ρ=0.51, $P<0.001$, see Appendix Fig. 1 and 2). This may indicate that the roots of smaller plant species did not reach deeper soil layers within 11 months.

Proportional similarity with regard to N uptake from shallow and deep soil significantly decreased with species richness (as a three-level factor and as a log-linear effect). However, it was the presence of interspecific competition rather than the number of interspecific competitors that mattered, since PS was similar in mixtures of two and four species (Fig. 1, Table 3). Moreover, species in mixtures were still relatively similar (mean = 0.86±0.01). PS was ≥0.65 at harvest 1, and ≥0.6 at harvest 2 and between all but one species pair at harvest 3 (PS=0.47 between *L. vulgare* and *P. lanceolata*, but 0.92 for the other replicate). The effect of both species richness and specific species pairs changed over time (across harvests).

Effects of species richness and community niche on community biomass and N content

Total community biomass increased with species richness (log-linear effect, $F_{1,19}$= 6.52, $P<0.05$), with average biomass in 2-species and 4-species mixtures amounting to 112 % and 116 % of the biomass in monocultures. However, the N content of the plant communities did not increase with species richness, since the increase in biomass was paralleled by a decrease in N concentration (at harvest 1 for instance, from an average of 1.04 % in monocultures to 0.96 and 0.94 % in 2-species and 4-species mixtures, respectively).

Community biomass also increased with the community niche (CN), predicted *a priori* from species N uptake in monocultures (Appendix Fig. 3, $F_{1,59}= 28.1$, $P<0.001$), independent of harvest time. Moreover, there was a significant positive relationship between CN and the N content of plant communities (Fig. 2, $F_{1,59}= 27.0$, $P<0.001$). This relationship between CN and community N content was stronger at harvests 2 and 3 than at harvest 1, indicated by a significant CN × harvest interaction. Although CN generally increased with species richness, CN of the mixtures never exceeded CN of the "best monoculture", indicating that CN is largely determined by species composition rather than species richness. However, at each harvest, a different species performed best at taking up N from both depths in monoculture (*L. perenne* at harvest 1, *H. lanatus* at harvest 2 and *L. vulgare* at harvest 3).

Discussion

Limited partitioning of soil N acquisition by depth

In our mesocosm experiment, we found limited evidence for differences in N resource niches and, in particular, vertical N partitioning as major drivers of positive biodiversity–productivity relationships in temperate grassland plant communities, although this has often been proposed (HilleRisLambers et al. 2004, Spehn et al. 2005). While we showed interspecific differences in the proportion of N derived from deep and shallow soil layers when species were grown in mixture, we found no effect of species richness on the total N uptake of plant communites.

Whereas linking N uptake from deep and shallow soil of individual species to N uptake of communities of varying species richness is a novel aspect of our study, we also found limited evidence for vertical N partitioning in our earlier studies. In a field experiment with plant communities comprising one, three or six species, we also showed higher N uptake from shallow soil than from deep soil, despite the different choice of soil depths for the shallow and deep layer (0–3 cm and 7–12 cm, von Felten et al. 2009). In that experiment, root

distributions of plant communities were unaffected by species richness (Wacker 2007). In a pot experiment with pots of different depths but constant volume, we found stronger net biodiversity and complementarity effects in shallow pots, indicating that horizontal root segregation might have been more important than the partitioning of rooting depths (von Felten and Schmid 2008). Moreover, when Mommer et al. (2010) used a DNA-based technique to compare species-specific root distributions, they found that although four-species mixtures of two common grasses and forbs produced significantly more roots than the monocultures of the same species, this overyielding of root biomass was not due to vertical niche differentiation. Instead, recent evidence suggests that pathogen-mediated root overproduction in species mixtures determines the patterns of community productivity and overyielding (de Kroon et al. 2012).

In general, water can be accessed earlier in the top soil after precipitation events, and as long as there is enough moisture, more N is available in the top soil where litter and atmospheric N inputs arrive and N turnover mostly occurs. This should favour shallow roots, at least under moist conditions, while deep roots are certainly beneficial under dry conditions. However, allocating a high proportion of resources to deep roots may be unfavorable, whenever competitors take more effective advantage of resources available in shallow soil layers and exclude deep-rooted species (Schenk 2008). Hence, although the trade-off between deep and shallow roots may prevent plants from exclusively exploiting the shallow soil horizon, there may be a general advantage of shallow vs. deep rooting.

Generalists rather than specialists

No differences in species N uptake from shallow and deep soil in monoculture combined with only slight differences in mixtures and high niche overlap (proportional similarity) suggest that the species used here are generalists rather than specialists with regard to N uptake from different soil depths. The decrease in proportional similarity (PS) of species pairs with regard to N uptake from shallow and deep soil with increasing species richness confirms our

findings in an earlier experiment (von Felten et al. 2009). Although decreasing PS could indicate a change from generalist behavior of species in monoculture to specialist behaviour in mixture, it did not result in higher N uptake of communities with increasing species richness. Had there been a "division of labour" or had subordinate species taken refuge in deep soil, we should have seen higher N uptake with increasing species richness. Instead, it seems that the decrease in PS was due to subordinate species being constrained to using N from shallow soil, precluded from growing deep roots by dominant species. Our results contrast with Berendse (1982), where the deep-rooting forb *P. lanceolata* derived more nutrients from deeper soil layers when grown with the competitively dominant grass *A. odoratum*.

Effects of composition vs. species richness

We found no effect of species richness on the N content of the plant communities, because the observed increase in biomass was paralleled by a decrease in N concentration. Lower N concentration in species-rich compared to species-poor communities was also shown in two large biodiversity experiments manipulating the species richness of temperate grassland plants from one to eight and nine species (van Ruijven and Berendse 2005, Roscher et al. 2008). van Ruijven and Berendse (2005) suggested that mixtures have a higher nitrogen use efficiency. This could be due to higher biomass of those species with larger biomass:N ratio, consistent with resource-competition theory (Tilman 1982 and 1990) predicting that mixtures are dominated by those species able to most efficiently acquire limiting resources. Alternatively, increasing species richness may have lead to shifts in biomass allocation, i.e., a larger fraction of stems (low N concentration) as opposed to leaves (high N concentration), indicating increased competition for light.

Moreover, increasing species richness was shown to increase shoot to root ratios due to constant root but increasing aboveground biomass (Bessler et al. 2009). This may indicate, that complementarity aboveground was more important than complementarity belowground.

While more diverse plant communities may be able to capture more light by building a more complex canopy, total N acquisition may be largely set by soil mineralisation rates, which may not change much with diversity. Nevertheless, a combination of above- and belowground niche complementarity may be needed to create strong effects of diversity on productivity.

Whereas we found no effect of species richness on the N content of plant communities, the latter could be predicted by the community niche, which heavily depended on species composition. This result is in line with other studies. For instance, just as at each harvest a single (but each time different) species aquired most N from both soil depths, dominant bunchgrasses acquired most of the N from all soil N pools in a study on invasion resistance, and there was no evidence suggesting that functional groups partitioned different soil N pools when biomass was incorporated (James et al. 2008). Similarly, in a study conducted in three temperate grasslands, Kahmen et al. (2006) found that total N uptake at the ecosystem level was determined by species or functional group identity, and thus by community composition rather than species richness. Indeed, Ashton et al. (2008) found enhanced plant N uptake in the presence of an interspecific neighbor, but since they used neighbor removal treatments, the effects of neighboring plants and plant density could not be separated.

Our results indicate that species composition was more important in determining biomass production and N content than was species richness. Also, the community niche of a mixture was never larger than that of the best monoculture. However, it is important to note that at each harvest, a different species performed best at taking up N from both depths. This raises the possibility of a temporal N niche (McKane et al. 1990) and implies an advantage over time for communities containing all three species instead of only one, consistent with the insurance hypothesis (McNaughton 1977, Yachi and Loreau 1999).

Potential caveats

There are some potential caveats of this study that should be noted. First, there was a comparatively small effect of species richness on productivity, and we do not know if, under

these conditions, species would have coexisted in the long run. We cannot exclude that we might have found stronger differentiation of species in N uptake in an experiment showing a larger biodiversity effect on biomass. Vice versa, since differentiation in N uptake depends on the combination of species, more differentiated species might have produced a larger biodiversity–productivity effect. Second, our experimental plant communities were certainly not fully established yet, especially in the first year of the experiment (harvests 1 and 2). Cardinale et al. (2007) found that biodiversity effects on productivity increased over time, because the magnitude of complementarity increased as experiments were run longer. In fact, some changes between the first and the second year, such as increased N uptake from deep soil by forbs, might have continued and could have led to different N uptake patterns in fully established plant communities. Moreover, the effect of species diversity on plant community N content increased over time in the Jena-Experiment (Oelmann et al. 2011), and we cannot exclude that the absence of such an effect in our experiment is due to its relatively short duration. Third, by measuring N partitioning by soil depth, we investigated only one specific type of resource partitioning. Although our results fit well with those of a field experiment where we used different depth ranges for the shallow and the deep soil layer (von Felten et al. 2009), a different choice of depths for the layers in the mesocosms may have led to different results. Also, other types of resource partitioning (e.g., temporal partitioning and partitioning of different chemical forms) are known and a combination of several may be necessary to explain positive biodiversity–productivity effects.

Conclusions

We conclude that our eight experimental species are generalists rather than specialists regarding N uptake from deep and shallow soil. Also, our results do not support the role of vertical N partitioning as an important driver of positive biodiversity effects on the N capture of mixtures in temperate grasslands. With regard to preferred N uptake from the

shallow soil layer and the decrease in proportional similarity of species at increasing levels of species richness, our results match those of an earlier experiment (von Felten et al. 2009). This is notable, since quite different experimental approaches were used (different species pools, mesocosms vs. field, organic ^{15}N label vs. tracer solutions, different choice of soil depths for the shallow and deep layer). Although we observed a decrease in proportional similarity of species (here with regard to N uptake from different soil depths and earlier with regard to N uptake from different soil depths and N forms) both experiments revealed only limited evidence for N partitioning.

A novel aspect of our mesocosm experiment is the specific test whether and how interspecific differences in N uptake from shallow and deep soil lead to enhanced community N uptake at higher levels of species richness. Based on the positive relationship between plant species richness and productivity repeatedly shown in grassland experiments, and differences in species' capacity to assimilate different chemical forms of N or to exploit different soil depths (see references in introduction), N partitioning has been proposed as an important driver. However, our results do not support this hypothesis, as we observed no effect of species richness on community N uptake. On the one hand, the increase in biomass with species richness was paralleled by a decrease in N concentration. On the other hand, community composition was more important than species richness in determining community N uptake.

Future studies on resource partitioning should incorporate multidimensional differentiation of resource niches among coexisting plant species, including spatial and temporal differentiation, and different nutrients. Moreover, theoretical models are needed to further assess the importance of resource partitioning as opposed to other mechanisms that can drive positive diversity–productivity relationships, such as reduced relative fitness differences (so called stabilizing effects, see Chesson 2000, Carroll et al. 2011).

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586 Appendices

587 **Appendix A** Table: Mean ^{15}N enrichment of aboveground biomass by harvest and
588 treatment and natural background ^{15}N.

589 **Appendix B** Table: Analysis of Variance for ^{15}N enrichment of aboveground biomass.

590 **Appendix C** Table: Analysis of Variance for ^{15}N uptake from deep soil (DF) in
591 monocultures

592 **Appendix D** Table: Analysis of Variance for the fraction of ^{15}N tracer taken up from deep
593 soil (DF) by populations of individual species grown in mixture.

594 **Appendix E** Figure: Fraction of ^{15}N uptake from deep soil (DF) and aboveground biomass
595 per species in all monocultures and mixtures.

596 **Appendix F** Figure: Relationship between the rank of each species in aboveground
597 biomass and its rank in the deep fraction DF (fraction of ^{15}N uptake from deep soil)
598 within mixture.

599 **Appendix G** Figure: Plant community biomass (above- and belowground) as a function of
600 the calculated community niche for each harvest.

Table 1: **Experimental Design:** Combinations of plant species composition and ^{15}N treatments. Plant communities were randomly assigned to boxes, that were subdivided in eight (2 × 4) compartments. ^{15}N treatments were randomly applied to sets of four adjacent compartments per box (treatment unit). We show the numbers of harvested compartments for one harvest of species pool AHLP, including *Arrhenaterum elatius* (A), *Holcus lanatus* (H), *Leucanthemum vulgare* (L) and *Plantago lanceolata* (P). Totals are given for species pool DLRT, including *Dactylis glomerata* (D), *Lolium perenne* (L), *Ranunculus acris* (R) and *Taraxacum officinale* (T), and for both pools together (overall). Since in this paper, only the ^{15}N labeled compartments are considered (deep and shallow), the numbers including compartments without ^{15}N are given in brackets. Three destructive harvests were conducted, at each of which one out of four compartments was harvested per treatment unit (all harvested ^{15}N labeled compartments: n=96 × 3=288). Note that we refer to all plants in one compartment as community, and to individual species within a compartment as populations. Nomenclature follows Lauber and Wagner (1998).

	Species composition											
Pool AHLP[a]	A	H	L	P	AH	AL	AP	HL	HP	LP	AHLP	all
Deep	2	2	2	2	2	2	2	2	2	2	4	24
Shallow	2	2	2	2	2	2	2	2	2	2	4	24
No ^{15}N	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(4)	(24)
Total	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	8 (12)	**48** (72)
Pool DLRT[b]	D	L	R	T	DL	DR	DT	LR	LT	RT	DLRT	all
Total	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	4 (6)	8 (12)	**48** (72)
Overall	Monocultures				2 Species						4 Species	all
Compartments	32 (48)				48 (72)						16 (24)	**96** (144)
Populations	32 (48)				96 (144)						64 (96)	192 (288)
Boxes	24 (24)				36 (36)						12 (12)	72 (72)

[a] *A. elatius* and *H. lanatus* are grasses, *L. vulgare* and *P. lanceolata* are forbs.

[b] *D. glomerata* and *L. perenne* are grasses, *R. acris* and *T. officinale* are forbs.

Table 2: Analysis of Variance for the fraction of ^{15}N tracer taken up from deep soil (DF) by populations of individual species grown in mixture (n=240). A linear mixed effects model with the random factors species pool, species composition (see Table 1) and compartment pair was used. Significance levels: . P<0.1, * P<0.05, ** P<0.01, *** P<0.001. See Appendix Table 4 for the same analysis but with species replaced by functional group (grasses vs. forbs).

	Deep Fraction DF				
Source	Num. df	Den. df	F	$P(>F)$	
Species richness (SR)	1	11	0.26	0.62	
Species	7	52	21.01	<.001	***
SR × Species	7	52	0.64	0.72	
Harvest (H)[a]	2	128	66.02	<.001	***
SR × H	2	128	2.67	0.07	.
Species × H	14	128	4.69	<.001	***
SR × Species × H	14	128	1.23	0.26	

[a] Harvests: August 2005, September 2005 and May 2006.

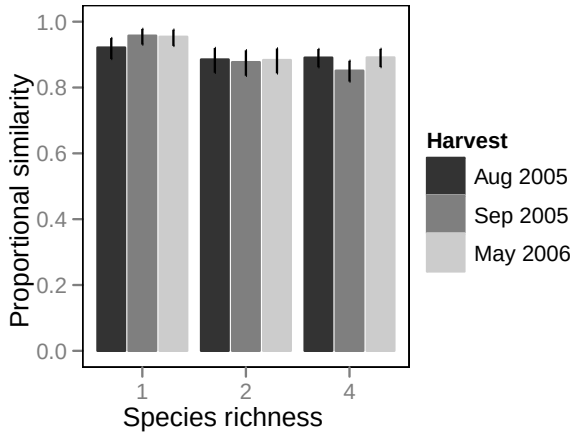
Table 3: Analysis of Variance for proportional similarity with regard to N uptake from shallow and deep soil between pairs of species (n=288). A linear mixed effects model with the random factors species pool, species composition (see Table 1) and specific pair was used. Specific pairs are the pairs of species (either two monocultures or two species within a mixture community) between which proportional similarity was calculated (n=96) at each of three harvests. Significance levels: . P<0.1, * P<0.05, ** P<0.01, *** P<0.001).

	Proportional similarity				
Source	Num. df	Den. df	F	$P(>F)$	
Species richness (SR)[a]	2	12	19.422	0.0002	***
Species pair (SP)	11	69	2.404	0.0137	*
Harvest (H)[b]	2	164	1.643	0.1967	
SR × Harvest	4	164	2.863	0.0251	*
SP × Harvest	22	164	2.246	0.0021	**

[a] Note that the difference between monocultures and mixtures would be highly significant if fitted instead of SR ($F_{1,13}$=39.21, P<0.001) and that the log-linear effect of SR is significant too ($F_{1,13}$=10.03, P=0.007).

[b] Harvests: August 2005, September 2005 and May 2006.

Figure Legends


1.0
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Proportional similarity
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Species richness
Harvest
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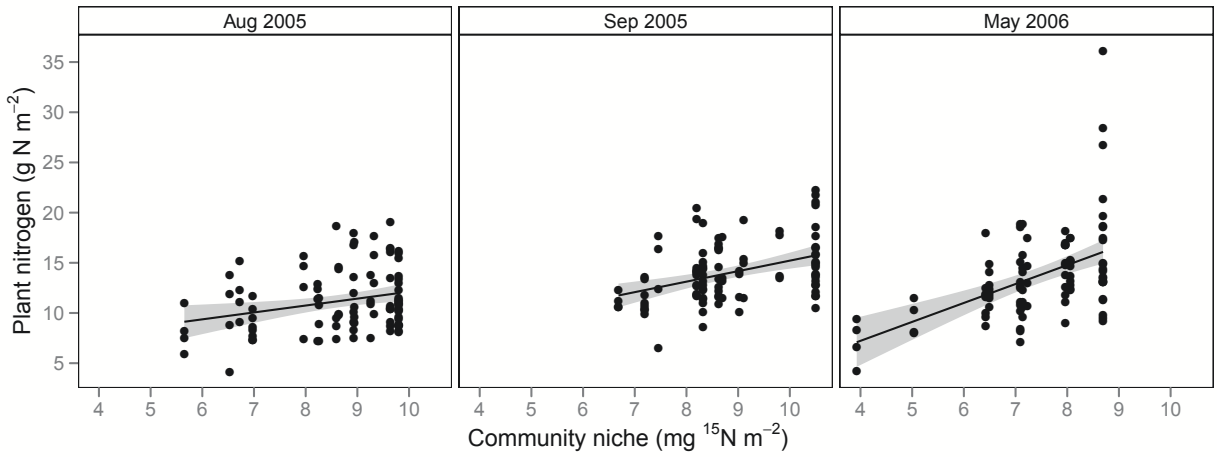
May 2006

Fig. 1:


Aug 2005
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Plant nitrogen (g N m^{-2})
Community niche (mg ^{15}N m^{-2})
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Fig. 2:

Appendix

Table 1: Mean ^{15}N enrichment of aboveground biomass by harvest and treatment (mean δ^{15}N in ‰±SE, n=96 populations for each mean). Natural background (BG) δ^{15}N is given as well (n=8 for harvests 1 and 2, and n=7 at harvest 3).

Harvest		^{15}N treatment		BG
		Shallow	Deep	
(1) August	2005	138.7±1.9	64.7±2.2	6.0±0.4
(2) September	2005	115.2±3.4	60.9±2.0	4.3±0.5
(3) May	2006	64.3±1.3	49.3±1.2	2.9±0.3

Table 2: Analysis of Variance for ^{15}N enrichment of aboveground biomass. A linear mixed effects model with the random factors treatment unit and compartment was used. Significance levels: . P<0.1, * P<0.05, ** P<0.01, *** P<0.001).

	^{15}N enrichment (δ^{15}N)				
Source	Num. df	Den. df	F	p(>F)	
Harvest	1	190	393.10	<.001	***
^{15}N Treatment	1	94	545.50	<.001	***
Harvest×^{15}N Treatment	1	190	169.13	<.001	***

[a] Harvests: August 2005, September 2005 and May 2006.

Table 3: Analysis of Variance for ^{15}N uptake from deep soil (DF) in monocultures. A linear mixed effects model with the random factors species pool and compartment pair was used. Significance levels: . P<0.1, * P<0.05, ** P<0.01, *** P<0.001).

	Deep Fraction DF				
Source	Num. df	Den. df	F	p(>F)	
Species	7	7	1.39	0.34	
Harvest[a]	2	16	17.79	<.001	***
Species×Harvest	14	16	1.07	0.44	

[a] Harvests: August 2005, September 2005 and May 2006.

Table 4: Analysis of Variance for the fraction of ^{15}N tracer taken up from deep soil (DF) by populations of individual species grown in mixture (n=240). A linear mixed effects model with the random factors species pool, species composition (see Table 1 in the original paper) and compartment pair was used. Significance levels: . P<0.1, * P<0.05, ** P<0.01, *** P<0.001). See Table 2 in the original paper for the same analysis but with species instead of functional group.

	Deep Fraction DF				
Source	Num. df	Den. df	F	$P(>F)$	
Species richness (SR)	1	11	1.59	0.23	
Functional group (FG)	1	64	6.90	0.01	*
SR×FG	1	64	1.73	0.19	
Harvest (H)[a]	2	152	57.92	<.001	***
SR×H	2	152	2.34	0.10	
FG×H	2	152	15.53	<.001	***
SR×FG×H	2	152	0.97	0.38	

[a] Harvests: August 2005, September 2005 and May 2006.

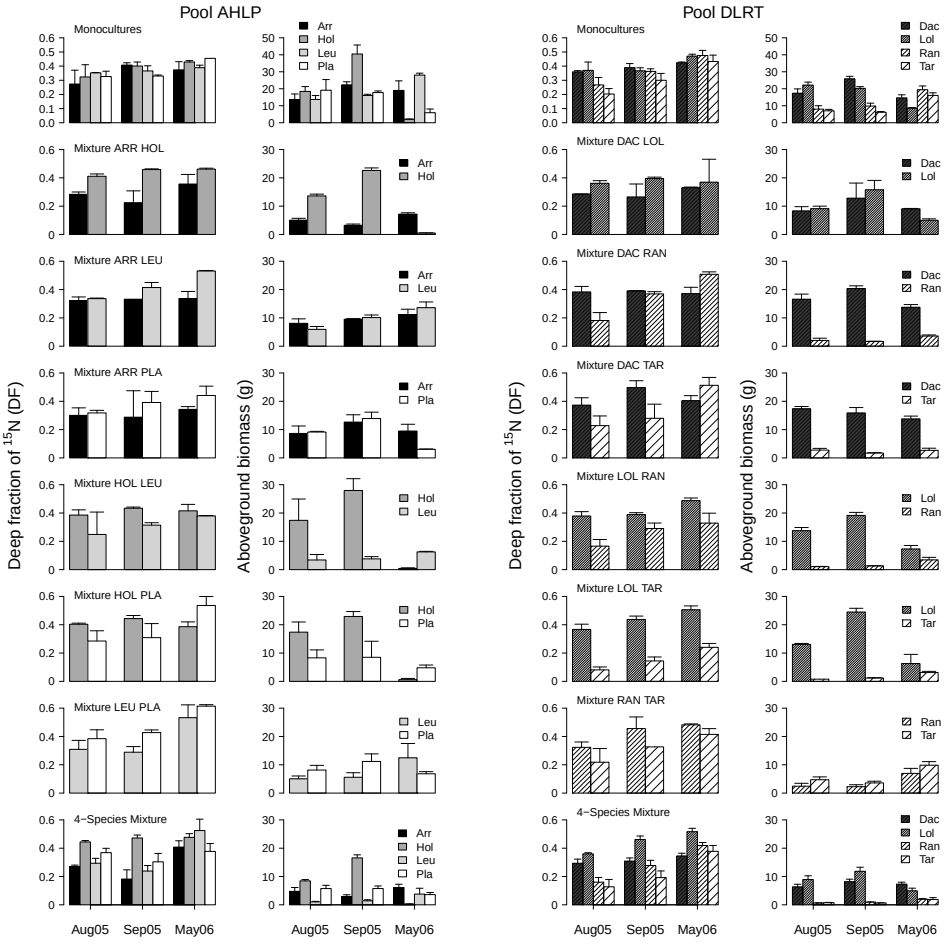

Fig. 1: Fraction of ^{15}N uptake from deep soil (DF) and aboveground biomass per species in all monocultures and mixtures of pool AHLP (left panels) and pool DLRT (right panels). Error bars show standard errors of the mean, monocultures and 2-species mixtures: n=2, 4-species mixtures: n=4 for each bar. See Table 1 (in the main text) for full species names.

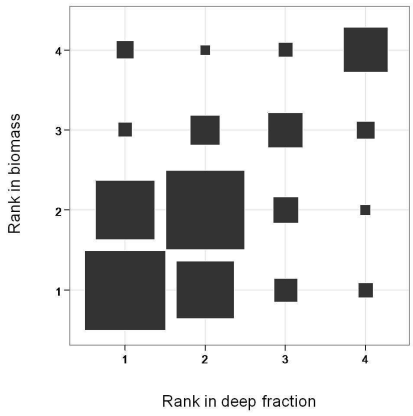

Fig. 2: Relationship between the rank of each species in aboveground biomass and its rank in the deep fraction DF (fraction of ^{15}N uptake from deep soil) within mixture. Example: A species contributes to the square-area in the lower left corner if it had the highest aboveground biomass within the mixture it belonged to, as well as the highest DF among all species in that mixture. The square-areas are proportional to the number of observations for each combination of ranks. The range of ranks is 1–2 and 1–4 in 2-species and 4-species mixtures, respectively.

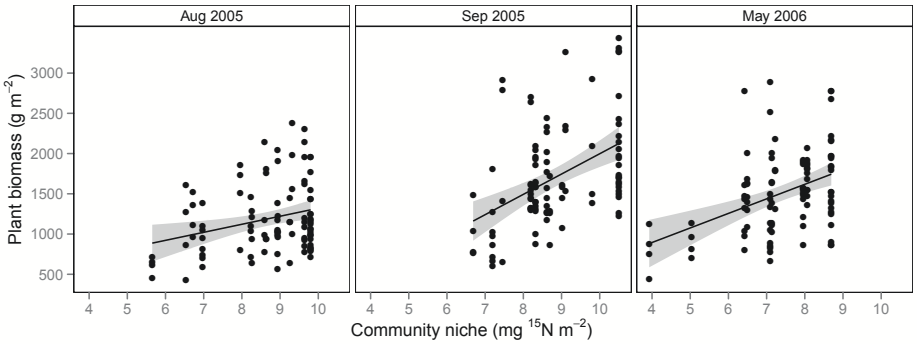

Fig. 3: Plant community biomass (above- and belowground) as a function of the calculated community niche for each harvest. The positive relationship is indicated by regression lines, including a 95 % confidence interval. Note that the community niche for the mixtures was determined *a priori* from ^{15}N uptake by individual plant species from deep and shallow soil (see Eq. 6 in the main text), whereas for the monocultures, it equals ^{15}N uptake from deep and shallow soil by one species only.